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Cloning of a human gene involved in cytochrome oxidase assembly by functional complementation of an *oxa1*$^-$ mutation in *Saccharomyces cerevisiae*

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ABSTRACT **The yeast nuclear gene *OXA1* is essential for cytochrome oxidase assembly, so that a null mutation in the *OXA1* gene leads to complete respiratory deficiency. We have cloned by genetic selection a human *OXA1* (*OXA1*Hs) cDNA that complements the respiratory defect of yeast *oxa1* mutants. The deduced sequence of the human protein shares 33% identity with the yeast OXA1 protein. The *OXA1*Hs cDNA corresponds to a single and relatively highly expressed gene. Oxygen consumption measurements and cytochrome absorption spectra show that replacement of the yeast protein with the human homolog leads to the correct assembly of cytochrome oxidase, suggesting that the proteins play essentially the same role in both organisms.**

Cytochrome oxidase, the terminal complex of the mitochondrial respiratory chain, has a critical role in both prokaryote and eukaryote cellular energy metabolism. It catalyzes the transfer of electrons from reduced cytochrome *c* to molecular oxygen, coupled to proton translocation across the inner mitochondrial membrane. Mammalian cytochrome oxidase has been purified as a complex of 13 subunits (1). The three largest subunits are encoded by the mitochondrial genome and represent, with hemes *a* and a_3 and two copper atoms, the catalytic core of the enzyme. The 10 other cytochrome oxidase polypeptides are encoded by the nuclear genome and imported into the mitochondria; they could play a role in regulating cytochrome oxidase activity. Most of the nuclear-encoded subunit genes have been cloned using oligonucleotides derived from the N-terminal sequence of their product (2).

Cytochrome oxidase from lower eukaryotes, like the yeast *Saccharomyces cerevisiae*, has been purified with a subunit composition resembling that of the mammalian enzyme—i.e., three mitochondrial-encoded polypeptides and nine nuclear-encoded polypeptides (3). The yeast cytochrome oxidase subunit genes have been cloned and sequenced, and the gene products exhibit a significant identity to their human counterpart, suggesting that the enzyme complex structure is conserved (4, 5). The fact that *S. cerevisiae* is a facultative aerobe has allowed the isolation of numerous respiratory-deficient mutants that are affected in nuclear genes encoding either cytochrome oxidase subunits or numerous other proteins essential for cytochrome oxidase biogenesis (6, 7). As the structural organization of cytochrome oxidase seems to be conserved from yeast to human, the human complex should similarly require a number of nuclear-encoded proteins controlling its biogenesis. However, to date there has been no report of the isolation of a human gene corresponding to one of these proteins. Therefore, we have used yeast as a genetic tool to search for these genes.

In our laboratory, we have analyzed several yeast genes encoding proteins controlling either the stabilization (8) and the splicing (9, 10) of mitochondrial transcripts or the assembly of enzyme complexes in the inner mitochondrial membrane (11, 12). This paper presents the cloning by functional complementation of the human homolog of one of these genes, *OXA1*,† which controls cytochrome oxidase assembly (12). We have chosen the *OXA1* gene for three reasons. (*i*) A null mutation in the *OXA1* gene leads to a complete respiratory deficiency. Thus, it should be possible to detect even a weak complementation by a human gene. (*ii*) The *OXA1* gene is relatively well expressed in yeast and we expected its human homolog to be well represented in an expression library. (*iii*) The sequence of the deduced OXA1 protein is similar to several putative prokaryotic proteins and shares some similarities with expressed sequence tags of eukaryotes, suggesting a certain conservation of this protein through evolution. Our results show that replacement of the yeast protein with the human homolog leads to the correct assembly of cytochrome oxidase, suggesting that the proteins play essentially the same role in both organisms.

MATERIALS AND METHODS

Yeast Strains and Media. *S. cerevisiae* strains used in transformations were BC79-6A (*MATα, ura3-52, his4-580, trp1-289, leu2-3, 112, oxa1-79*; a gift of R. O. Poyton, University of Colorado, Boulder) and NBT1 (*MATα, ade2-1, ura3-1, his3-11, 15, trp1-1, leu2-3, 112, can1-100, oxa1::LEU2*) (12).

Yeast media were as follows: complete glucose medium, 1% yeast extract/1% Bacto Peptone/2% (wt/vol) glucose (as a fermentable carbon source)/adenine (20 mg/liter); minimal glucose medium, 0.67% yeast nitrogen base without amino acids/2% glucose; glycerol medium, 1% yeast extract/1% Bacto Peptone/0.05 M sodium potassium phosphate, pH 6.25/2% glycerol (as a nonfermentable carbon source). Auxotrophy supplements were added as required (each at 20 mg/liter).

Yeast Transformations. The D98-H2 HeLa cell cDNA library was constructed in vector pFL61 (13, 14) and amplified as described (15). pFL61 contains the replication origin of the yeast 2 μm plasmid, the *URA3* gene, and the promoter and terminator of the yeast phosphoglycerate kinase gene for the expression of foreign cDNAs in *S. cerevisiae*. Cells from an exponential-phase culture of the BC79-6A strain grown in complete medium were transformed with the HeLa cell cDNA library by the lithium acetate procedure (16). Plasmid

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Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. X80695).

DNAs (YEp61/Hs1 to Hs10) from yeast transformants were extracted (17) and amplified in *Escherichia coli* by standard procedures (18). Retransformations of yeast with purified plasmids were performed by the one-step technique (19).

DNA Sequencing and Sequence Analysis. The cDNA carried by the plasmid YEp61/Hs2, isolated from the HeLa cell cDNA library by complementation, was subcloned into M13mp18 and M13mp19 and totally sequenced by the dideoxynucleotide chain-termination method using the Sequenase kit (United States Biochemical). To ensure that the nine other plasmids (YEp61/Hs1, Hs3 to Hs10) were carrying the same gene, the 5′ ends of the nine cDNAs were sequenced and were shown to differ by only 1 or 2 bp at their beginning. Sequence analyses were carried out using the University of Wisconsin Genetics Computer Group package (20).

Southern and Northern Blot Analyses. Total genomic DNA of HeLa cells was digested with restriction enzymes. Fragments were separated through a 0.7% agarose gel, transferred, and hybridized (18).

Total RNAs, a gift of Y. Lécluse (Institut G. Roussy, Villejuif, France), were resolved by electrophoresis through a 1.2% agarose/formaldehyde gel, blotted onto a Hybond-C extra membrane, and hybridized in 5× SSPE (1× SSPE = 0.15 M NaCl/8.8 mM NaH_2PO_4/1 mM EDTA, pH 7.4)/0.1% SDS/5× Denhardt's solution/50% (vol/vol) formamide/sheared salmon sperm DNA (100 μg/ml). The membrane was washed at 65°C in 2× SSPE/0.1% SDS. Quantification of the signals was performed by direct phosphor imaging of the hybridized filter (Molecular Dynamics).

The human *OXA1* probe (*OXA1*Hs) was a gel-purified 832-bp *Bam*HI–*Eco*RI fragment (see Fig. 1). The human actin probe, a gift of V. Lamour (Institut G. Roussy, Villejuif, France), was a gel-purified 1200-bp fragment corresponding to the 3′ end of the actin cDNA. The probes were α-^{32}P-labeled by random-primer DNA labeling.

Growth Rate Measurements. Yeast cell growth was followed by density measurements using a Klett–Summerson photoelectric colorimeter. Doubling time during exponential growth was calculated from a plot of logarithm (cell number) vs. time.

Determination of Oxygen Consumption Rates. The oxygen consumption of yeast cells was measured with a Clark electrode (Gilson oxygraph). Cells were grown to late exponential phase, pelleted for 5 min at 1500 × *g*, and washed once with Ringer's solution. The cells were resuspended in 50 mM potassium phthalate buffer (pH 4.5) preheated at 30°C. Oxygen consumption was measured after addition of 2% ethanol as a respiratory substrate. The uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was added at a final concentration of 25 μM.

Cytochrome Absorption Spectra. Whole cell absorption spectra were recorded at low temperature as described (21) using a Cary 219 spectrophotometer. The cytochromes were fully reduced by addition of a few milligrams of sodium dithionite.

RESULTS

A Human cDNA Complements the *oxa1-79* Mutation in *S. cerevisiae*. Cells carrying a mutated *oxa1* gene are respiratory-deficient and, thus, do not grow on complete medium containing a nonfermentable carbon source such as glycerol (12). To select a human cDNA that complements this respiratory defect, the strain BC79-6A, carrying the *oxa1-79* mutation, was transformed with a HeLa cell cDNA library constructed in the vector pFL61 (13, 14). About 250,000 URA$^+$ transformants were selected on minimal glucose medium lacking uracil for plasmid maintenance and replica-plated on glycerol medium. Ten respiratory-competent transformants were able to grow on glycerol medium after a 12-day incubation at 28°C. The 10 plasmids recovered from these respiratory-competent transformants complemented the *oxa1-79* mutation after retransformation of BC79-6A, although the transformants grew slowly on glycerol medium (see below). All the plasmids presented the same restriction profile (data not shown), suggesting that they carried the same cDNA.

The Cloned Human cDNA Encodes a Homolog of the OXA1 Protein. The sequence of the longest cDNA (1551 bp long) is presented in Fig. 1 and reveals a long open reading frame able to encode a protein of 435 amino acids. Three in-frame ATG codons are close together at the beginning of the open reading frame; the first and second are in contexts that follow Kozak's rules for initiation (23). The nine other complementing cDNAs also contain the three ATG codons and only differ by 1 or 2 bp at their 5′ ends. To search for longer cDNA fragments, we have carried out a PCR amplification of the

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   ggcaaaATGGCGATGGGACTAATGTGCGGA CGCCGGGAGCTTCTGCGCTTGCTACAGTCC GGGCGTCGGGTCCACAGCGTCGCAGGGCCC TCGCAATGGCTTGGGAAACCGCTGACCACA  120
 1       M  A  M  G  L  M  C  G   R  R  E  L  L  R  L  L  Q  S   G  R  R  V  H  S  V  A  G  P   S  Q  W  L  G  K  P  L  T  T

   CGGCTCCTATTCCCAGTAGCCCCGTGCTGC TGTCGCCCACACTACCTCTTCCTTGCGGCT TCCGGCCCCCGCAGCCTCAGTACCTCTGCT ATCTCTTTTGCAGAAGTCCAGGTTCAGGCC  240
39 R  L  L  F  P  V  A  P  C  C  C  R  P  H  Y  L  F  L  A  A   S  G  P  R  S  L  S  T  S  A   I  S  F  A  E  V  Q  V  Q  A

   CCTCCTGTTGTTGCTGCAACTCCCTCACCC ACAGCAGTACCTGAGGTGGCTTCTGGAGAG ACTGCAGATGTAGTCCAAACTGCTGCAGAG CAGAGCTTCGCTGAACTGGGGCTGGGGTCA  360
79 P  P  V  V  A  A  T  P  S  P   T  A  V  P  E  V  A  S  G  E   T  A  D  V  V  Q  T  A  A  E   Q  S  F  A  E  L  G  L  G  S

   TACACCCCAGTGGGACTGATCCAGAATTTA CTGGAATTTATGCATGTTGATCTGGGCCTA CCTTGGTGGGGGGCCATTGCTGCATGTACA GTCTTTGCCCGCTGCCTGATTTTTCCTCTC  480
119 Y  T  P  V  G  L  I  Q  N  L   L  E  F  M  H  V  D  L  G  L   P  W  W  G [A  I  A  A  C  T] V  F  A  R  C  L  I  F  P  L

   ATCGTGACGGGCCAGCGAGAGGCAGCCAGG ATCCACAATCACTTGCCAGAGATCCAGAAG TTTTCCAGTCGAATCAGAGAGGCCAAGTTA GCAGGAGACCATATTGAGTATTACAAGGCT  600
159 [I  V  T  G  Q] R  E  A  A  R   I  H  N  H  L  P  E  I  Q  K   F  S  S  R  I  R  E  A  K  L   A  G  D  H  I  E  Y  Y  K  A

   TCCTCGGAGATGGCACTTTACCAGAAAAAA CATGGTATTAAACTCTATAAACCTCTCATT CTCCCTGTGACTCAGGCCCCAATCTTCATC TCCTTCTTCATTGCTTTGAGAGAGATGGCC  720
199 S  S  E  M  A  L  Y  Q  K  K   H  G  I  K  L  Y  K  P [L  I   L  P  V  T  Q  A  P  I  F  I   S  F  F  I  A  L  R] E  M  A

   AACCTTCCTGTGCCCAGCCTGCAGACAGGT GGCCTCTGGTGGTTCCAGGATCTCACGGTA TCCGATCCCATCTACATATTACCACTGGCA GTCACTGCTACAATGTGGGCTGTTCTTGAG  840
239 N  L  P  V  P  S  L  Q  T  G   G  L  W  W  F  Q  D  L  T  V   S  D  P [I  Y  I  L  P  L  A  V  T  A  T  M  W  A  V  L  E]

   CTAGGTGCTGAGACAGGTGTGCAAAGTTCT GACCTTCAGTGGATGAGAAATGTCATCAGA ATGATGCCCCTGATAACCTTGCCCATAACC ATGCATTTCCCCACGGCAGTGTTTATGTAC  960
279 [L] G  A  E  T  G  V  Q  S  S   D  L  Q  W  M  R  N  V  I  R   M  M  P  L [I  T  L  P  I  T  M  H  F  P  T  A  V  F  M  Y]

   TGGCTCTCCTCCAATTTGTTTTCCCTGGTC CAAGTATCCTGTCTCCGGATTCCAGCAGTA CGCACTGTACTTAAAATCCCCCAGCGTGTT GTACATGACCTGGACAAATTACCTCCACGG 1080
319 [W] L  S  S  N  L  F  S [L  V   Q  V  S  C  L  R  I  P  A  V   R  T  V  L  K] I  P  Q  R  V   V  H  D  L  D  K  L  P  P  R

   GAAGGCTTCCTAGAGAGCTTCAAAAAAGGC TGGAAAAATGCTGAAATGACGCGTCAGCTG CGAGAGCGTGAACAACGCATGCGGAATCAG TTGGAGCTAGCAGCCAGGGGTCCTTTACGA 1200
359 E  G  F  L  E  S  F  K  K  G   W  K  N  A  E  M  T  R  Q  L   R  E  R  E  Q  R  M  R  N  Q   L  E  L  A  A  R  G  P  L  R

   CAGACCTTTACCCACAACCCTCTCCTACAA CCTGGAAAGGATAACCCTCCCAATATCCCT AGCAGCAGCAGCAAACCAAAGTCAAAGTAT CCCTGGCACGACACACTTGGCTGActtatg 1320
399 Q  T  F  T  H  N  P  L  L  Q   P  G  K  D  N  P  P  N  I  P   S  S  S  S  K  P  K  S  K  Y   P  W  H  D  T  L  G  *

   ttctgtgcgcattctggcaggaatttctgtc tcttcagagactcatcctcaaaacaagact tgacactgtgtccttgccccagtcctagga actgtggcacacagagatgttcatttttaaa 1440
   aacggatttcatgaaacactcttgtactta tgtttataagagagcactgggtagccaagt gatcttcccattcacagagttagtaaacct ctgtactacaaaaaaaaaaaa             1451
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FIG. 1. Nucleotide and deduced amino acid sequences of the longest *OXA1*Hs cDNA. The *Bam*HI and *Eco*RI restriction sites used for the probe preparation are underlined. A potential polyadenylylation signal is double underlined. Five hydrophobic stretches were determined according to both the ALOM and Kyte and Doolittle programs (20, 22) and are boxed.

cDNA library, using a primer specific for the 5′ end of the *OXA1*Hs cDNA and a primer specific to the library vector. A single fragment corresponding to the cloned cDNAs was found, indicating that no longer *OXA1*Hs cDNA is present in the library.

The human cDNA nucleotide sequence does not present any strong similarity to that of the yeast *OXA1* gene. However, the deduced human protein sequence shares 33% identity with the 402-amino acid yeast OXA1 protein (Fig. 2*A*). Thus the human cDNA we have cloned by functional complementation of the *oxa1-79* mutation appears to encode the human homolog of the OXA1 protein. We have called this cDNA *OXA1*Hs to distinguish it from the yeast *OXA1* gene. Like its yeast counterpart (12), comparison of the OXA1Hs protein sequence to databases revealed a significant similarity with the *Bacillus subtilis spoIIIJ/orf261* gene product whose absence blocks sporulation (25, 26), with the corresponding *E. coli* and *Pseudomonas putida* proteins, whose functions are unknown (26), and with eukaryotic expressed sequence tags of unknown functions (see Fig. 2*B* and *Discussion*).

The *OXA1*Hs cDNA Corresponds to a Single and Relatively Highly Expressed Gene. Southern blot analysis (Fig. 3*A*) shows that the *OXA1*Hs probe hybridizes at high stringency to a single band of human genomic DNA in each restriction endonuclease digestion tested, including those digestions that extend on each side far away (20 kb and 6.6 kb) from the *OXA1*Hs open reading frame. This confirms that the *OXA1*Hs cDNA is of human origin and indicates that there is only one human *OXA1*Hs locus. Moreover, the 0.8-kb *Bam*HI–*Eco*RI *OXA1*Hs probe reveals a 2.0-kb *Bam*HI–*Eco*RI genomic DNA fragment, suggesting that the genomic fragment contains intronic sequences. As expected from nucleotide sequence comparison, we have not detected any cross hybridization of the *OXA1*Hs probe to yeast genomic DNA (data not shown), implying that the *OXA1*Hs cDNA could not have been cloned by sequence complementarity.

Northern blot analysis of total RNAs from various cell lines shows a unique transcript of roughly 2 kb (Fig. 3*B*). PhosphoImager quantification of *OXA1*Hs and human actin hybridization signals revealed that *OXA1*Hs, like its yeast counterpart, is expressed at a relatively high level (2–4% of the actin mRNA) in all human cell lines tested. The comparison of the size of the cloned cDNAs (≈1550 bp) and the estimated size of the transcript (≈2 kb) suggests that the cloned cDNAs do not correspond to the full length of the mRNA. However, the 5′ missing part of the *OXA1*Hs cDNAs, if any, is not essential for the production in yeast of a functional protein since all the cloned cDNAs complement the *oxa1-79* mutation.

The *OXA1*Hs cDNA Restores the Assembly of the Cytochrome $a+a_3$ and the Respiration in the *oxa1* Null Mutant. We have investigated whether the *OXA1*Hs cDNA was able to complement a null mutation in the yeast *OXA1* gene. The yeast strain NBT1, which has an *oxa1*::*LEU2* disruption (12), was transformed with the YEp61/Hs2 plasmid carrying the cloned *OXA1*Hs cDNA or with the YEpNB6 plasmid carrying the wild-type yeast *OXA1* gene. As shown in Fig. 4*A*, the *OXA1*Hs transformants are respiratory competent; however, they grow more slowly on glycerol medium than the corresponding yeast *OXA1* transformants. As expected, the transformants carrying the control vectors do not grow at all on this nonfermentable medium. Thus, the *OXA1*Hs cDNA product is able to replace a total absence of its yeast homolog.

As the complementation of both the *oxa1-79* and the *oxa1* null mutation by the *OXA1*Hs cDNA appeared to be partial, we have compared three respiratory parameters in the *OXA1*Hs and *OXA1* transformants. (*i*) The respiratory

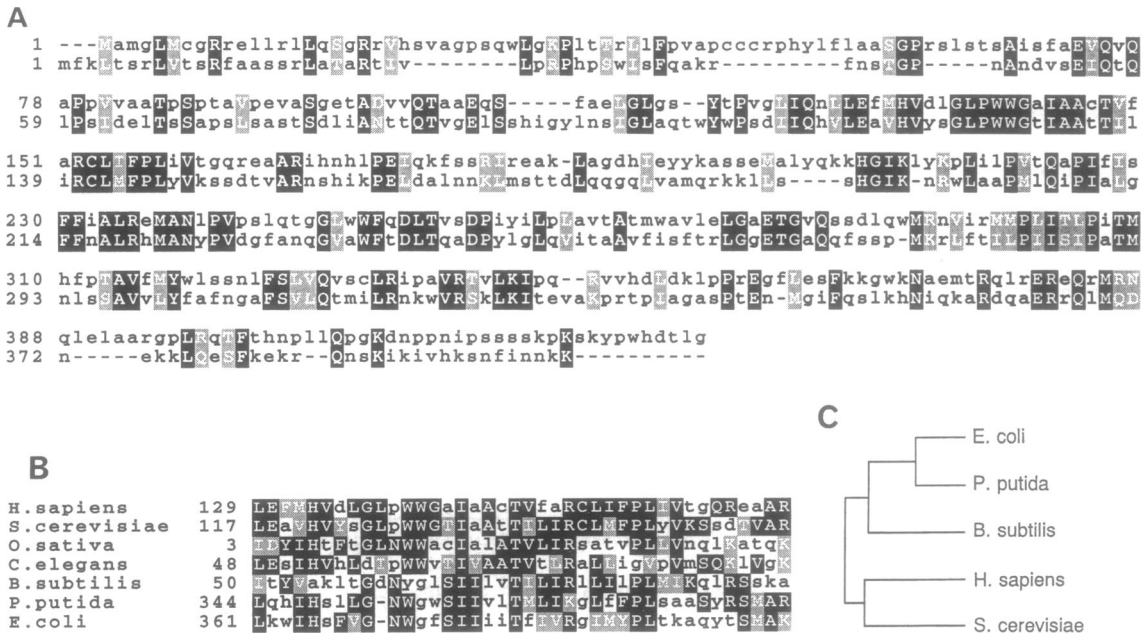

FIG. 2. Alignment of the human OXA1Hs deduced protein sequence with the yeast OXA1 and other eukaryotic and prokaryotic protein sequences. The OXA1Hs protein was aligned with its homologs by using the MULTAL/CLUSTAL program (gap weight, 10; length weight, 10; ref. 20) based on the Dayhoff's matrix. Nonconserved residues are in lowercase type. Uppercase type is used for similar residues (white type on a shaded background) and for identical residues (white type on a black background). (*A*) Alignment of the whole *Homo sapiens* and *S. cerevisiae* deduced proteins. The upper line corresponds to the human sequence and the lower line corresponds to the yeast sequence. (*B*) Alignment of a 40-amino acid region of *H. sapiens*, *S. cerevisiae* (12), *Oriza sativa* (GenBank accession no. D22376), *Caenorhabditis elegans* (24), *B. subtilis* (25, 26), *P. putida* (26), and *E. coli* (GenBank accession no. L10328) deduced proteins. The number of the first residue presented for each sequence is indicated. For *O. sativa* and *C. elegans* expressed sequence tags, number 1 corresponds to the beginning of the sequence available in GenBank and not to the first residue of the protein. (*C*) Dendogram of a 252-amino acid region of the *H. sapiens* protein (positions 82–333) aligned with its *S. cerevisiae* and bacterial homologs by the PILE-UP program (20). The dendogram should not be taken to represent evolution distances but only the degree of resemblance.

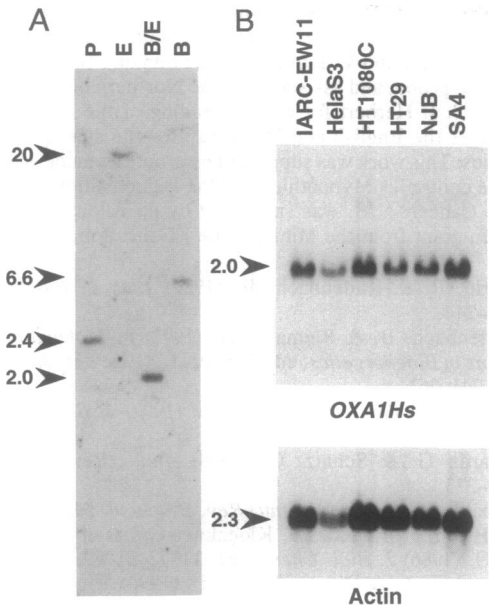

FIG. 3. (*A*) Southern blot analysis of genomic human DNA. HeLa cell DNA (23 μg) was digested with *Pst* I (P), *Eco*RI (E), *Bam*HI/*Eco*RI (B/E), or *Bam*HI (B) and hybridized to the *OXA1*Hs probe. The autoradiograph presented is from a 6-day exposure. λ DNA digested with *Sty* I was used for size calibration in kb. (*B*) Northern blot analysis of human RNA. Total RNAs (20 μg) from various cell lines were hybridized successively to the *OXA1*Hs probe and to the actin probe. IARC-EW11, Ewing sarcoma cells; HeLaS3, HeLa cells; HT1080C, fibrosarcoma cells; HT29, colon adenocarcinoma cells; NJB, neuroblastoma cells; SA4, liposarcoma cells. Exposure times of the autoradiographs presented were 6 h for the *OXA1*Hs probe and 1 h for the actin probe. Transcript sizes (indicated in kb) were calculated from the migration of rRNAs, visualized by staining with ethidium bromide.

growth in liquid glycerol medium was followed for 24 h. The *OXA1*Hs transformants grow more slowly than the *OXA1* transformants and the maximal growth level is four times lower (Fig. 4*B*). (*ii*) We have examined the oxygen consumption of cells grown in glycerol medium and tested whether the uncoupler CCCP (a protonophore) could stimulate this respiration. The CCCP-stimulated rate divided by the rate in the absence of CCCP varies inversely with respect to the relative proton permeability of the membrane. The *OXA1*Hs transformants respire better than the *OXA1* transformants; however, there is only a little increase of the respiratory capacity when CCCP is added, showing an increased proton permeability of the membrane in the *OXA1*Hs transformants. (*iii*) We have analyzed the cytochrome content of both the *OXA1*Hs and *OXA1* transformants by recording the whole cell absorption spectra. We had reported (12) that the disruption of the *OXA1* gene led to a complete lack of the cytochrome $a+a_3$ absorption peak, characteristic of cytochrome oxidase. Fig. 4*C* shows that the *OXA1*Hs and *OXA1* transformants display a normal cytochrome $a+a_3$ absorption peak. Thus, in the presence of the *OXA1*Hs cDNA product, the yeast cytochrome $a+a_3$ is correctly assembled, respiration occurs, but some proton leakage exists.

DISCUSSION

We have cloned a human *OXA1*Hs cDNA that complements the respiratory defect of *oxa1* yeast mutants and encodes a protein sharing a significant similarity (33% identity) with the 402-residue yeast OXA1 protein. The highest similarity between the yeast and the human protein (40% identity) is

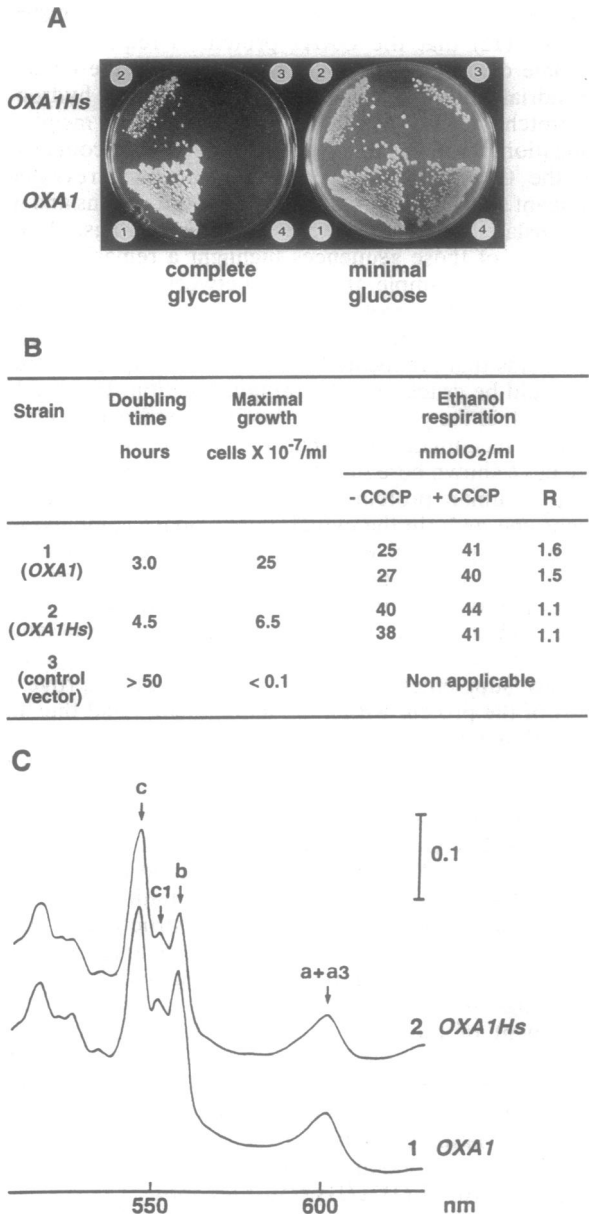

FIG. 4. Complementation of a yeast *oxa1* null mutation with the human *OXA1*Hs cDNA. (*A*) Growth of the transformants on a nonfermentable medium. Transformants were streaked on minimal glucose medium lacking uracil for plasmid selection (right plate) and replica-plated onto glycerol medium (left plate). Quadrants: 1, strain NBT1 (*oxa1*::*LEU2*) transformed with the plasmid YEpNB6 carrying the *OXA1* yeast gene cloned in pFL44L; 2, NBT1 transformed with the plasmid YEp61/Hs2 carrying the *OXA1*Hs human cDNA cloned in pFL61; 3, NBT1 transformed with the control expression vector pFL61; 4, NBT1 transformed with the control vector pFL44L. Photographs were taken after a 4-day incubation at 28°C. (*B*) Growth rate and oxygen consumption measurements. Strains 1, 2, and 3 (as in *A*) were grown in glycerol medium. For strains 1 and 2, oxygen consumption values from two experiments are given as nmol of O_2 per ml for 5×10^7 cells in presence or in absence of the uncoupler CCCP, the solubility of oxygen in an aqueous solution being estimated as 222 μM at 30°C. R indicates the ratio of the CCCP-stimulated rate divided by the rate in the absence of CCCP. (*C*) Cytochrome absorption spectra. Spectra were recorded in liquid nitrogen from whole cells grown on glycerol medium. Strains 1 and 2 are as in *A*. Absorption maxima expected for the α bands of cytochrome $a+a_3$, b, c_1, and c are at 602, 558, 552, and 546 nm, respectively, and are indicated by arrows.

observed in their central region, including five hydrophobic stretches (12) that are present in the yeast enzyme and

conserved in the human enzyme (Fig. 1). In yeast, we have proposed (12) that the OXA1 protein is required for cytochrome oxidase assembly, which occurs in the inner mitochondrial membrane. The conservation of these hydrophobic stretches is an argument in favor of the membrane localization of the OXA1 protein. Like its yeast counterpart (12), the OXA1Hs deduced protein sequence revealed a significant similarity with three prokaryotic proteins and with two translated eukaryotic expressed sequence tags. Multiple alignments of these sequences highlight a remarkable conservation. For example, the region between residues 129 and 168 of the OXA1Hs protein, presented in Fig. 2*B*, displays a very high level of similarity including two strictly conserved amino acids that are located in the first hydrophobic stretch and should be crucial for the protein function. Finally, Fig. 2*C* shows that the yeast OXA1 protein sequence more closely resembles its human counterpart than its bacterial homologs.

We have shown here that replacement of the yeast protein with the human protein permits the correct assembly of cytochrome $a+a_3$ in the cytochrome oxidase complex, thus leading to respiration. However, this replacement seems to increase the proton permeability of the membrane. It is unlikely that the specific defect in proton translocation could be due to the fact that the cloned cDNAs are not full length, although we cannot exclude the possibility that the presence of a few supplementary amino acids would improve the import of the protein within the mitochondria and thus help its functioning. Alternatively, the membrane permeability could be more sensitive than the electron transfer to some minor conformational changes induced by the replacement of the yeast protein with the human protein. We have proposed (12) that the OXA1 protein may be required for the assembly of one or several of the three mitochondrially encoded subunits. It is interesting to note that a mutation in subunit I of ubiquinol oxidase of *E. coli* (which is structurally similar to the cytochrome $a+a_3$ type oxidase of eukaryotes) results in a dramatic decrease in proton pumping activity but with little change in electron transfer activity (27). Similarly, the replacement of the yeast OXA1 protein with the human protein could disturb the integration of the yeast subunit I in the membrane, leading to some defect in proton translocation. This proton leakage could be responsible for a decrease in phosphorylation efficiency and thus in energy production, explaining the reduction of both the growth rate and the maximal growth of *OXA1*Hs transformants.

In conclusion, we stress that cytochrome oxidase assembly is an intricate process involving interactions of several proteins, two hemes, and two copper atoms. Thus, it is very encouraging to have cloned a protein by heterologous complementation that is involved in such a complicated process. This approach should permit the isolation of other human genes controlling various steps of respiratory complex formation, taking advantage of the large collection of respiratory-deficient yeast mutants available in several laboratories. Mutations in such genes could be responsible for human diseases characterized by respiratory deficiencies—e.g., due to cytochrome oxidase defects (28). The yeast selection strategy should facilitate the definition of which step of the cytochrome oxidase biogenesis is controlled by the cloned human gene.

Note Added in Proof. G. Michaelis has communicated to us that the yeast *OXA1* gene (ref. 12) has been independently identified as yeast *PET1402* (ref. 29; GenBank accession no. X74456).

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